Appendix A

Diversity amongst anti-hapten antibodies

This appendix outlines some of the studies that have been performed to determine the extent of variation between different antibody molecules raised against a common, simple determinant. Four different examples are provided, in which four different haptens were used as the immunogen. Copies of the respective academic publications are provided herewith.

These and other studies demonstrate that widely diverse sequences are generated during the immune response to any particular antigen, due to a combination of V, D, and J region selection, VJ and VDJ splicing, and somatic mutation.

The operation of these events make it essentially impossible to identically reproduce an antibody with a somatically mutated sequence by immunizing a second animal. Clones producing antibody molecules with identical sequences have been described only: a) when based on a nearly *un-mutated germ-line sequence*, in which case the antibody is observable during the primary response; or b) when obtained from the *same* animal, in which case the responsible antibody-producing cells are derived from the same clonal progenitor.

Example 1:

Nahmias et al. (1988, J. Immunol. 140:1304) raised a panel of 14 monoclonal antibodies against the ß-adrenergic hapten alprenolol, also using the same mouse strain. The 14 antibodies utilized at least seven V_L, four J_L, eight V_H, and three J_H genes (Table I), and also demonstrated extensive splicing and mutational diversity (Figures 3-5). Only three pairs of hybridomas used the same H and L chain gene rearrangements; each related pair was obtained from a single mouse and was apparently derived from the same clone (page 1308, ¶ 3).

Example 2:

Stenzel-Poore et al. (1989, J. Immunol. 143:4123) raised a panel of monoclonal antibodies against phosphocholine (PC) in BALB/c mice and F1 hybrids. Fourteen monoclonal antibodies were selected from the high affinity Group II anti-PC that emerge in the secondary

response. The 14 antibodies utilized four V_L, six J_L, six to eleven V_H, four J_H genes, and more than five D genes (Table III). The antibodies each had an average of 3.6 replacement mutations in the heavy chain and 3.1 replacement mutations in the light chain (Table V). Here, as in other studies, the mutations were found *throughout the variable region* – they were enriched in the complementarity determining region (CDR), but also occurred in the framework (Table V).

Example 3:

Blier et al. (1987, J. Immunol. 139:3996) obtained monoclonal antibodies specific for 4-hydroxy-3-nitrophenyl acetate (NP). Twenty eight hybridomas were obtained during the secondary response in the *same mouse*. Fourteen were derived from different clones. Amongst the 14 families, about 3 different V_H, 3 different D and 3 different J_H were used (Table I). Nine families used the same V_H and D region genes, but were all spliced differently to create differences in the CDR4 region (Figure 1). Amongst the 28 antibody panel, there was an average of *8.1 amino acid replacements in each heavy chain variable region* (Table III). On average, *2.5 replacements had occurred after divergence of members of each clone family* (Table III). This indicates that somatic mutation is an *ongoing* process within B-cell clones during the immune response.

Example 4:

Leahy et al. (1988, Proc. Natl. Acad. Sci. USA 85:3661) raised a panel of 12 monoclonal antibodies against a DNP spin-label hapten. The mouse strain used was BALB/c, the same as was used in the disclosure of the instant application to develop 1A7. The amino acid sequences of both the heavy and light chains of the Leahy panel demonstrate that different clones are derived from different germ-line V genes, exhibit junctional diversity around the splice sites, and show mutational divergence from common germ-line precursor sequences. As a result, the sequences are dramatically different amongst the antibodies. These sequences are reproduced below:

```
                -15       -5    1         10        20        30   35 36
                 |         |    |         |         |         |    AB
ANO2       MRVLILLWLFTAFPGILSDVQLQESGPGLVKPSQSQSLTCTVTGYSITSDYAWN WI
ANO1       .K..S..Y.L..I.....................L....S.....G.Y..  ..
ANO3       .K..S..Y.L..I.....................L....S.....G.Y..  ..
ANO7       .......C.....................D.....L.....G.S.H  ..
ANO5     ME.HW.F.F..SVTA.VH.QF.P.Q..AE.A..GA.VKMS.KAS..TF..YWMH   .V
ANO6     ME.HW.F.F..SVTA.VH.Q..P.Q..AE.A..GA.VKMS.KAS...F.RYWMH   .V
ANO4     MGWSW.F.F.LSGTA.VHCQI..KQ...E....GA.VKIS.KAS...F.DY.IN   .V
ANO9     MGWSY.I.F.VATATDVH.Q....QP.AE....GA.VK.S.KAS..TF..YWMH   .V
AN11       MSW.F.F.LSGTA.VH.E....Q...E..R.GA.VKMS.KAS..TF..YVMH   .V
AN12     MEWNWVV.F.LSLTA.VYAQG.M.Q..AE....GA.VK.S.KTS.FTFR.S.IG   .L
ANO8     MEWLWN..F.MA.AQS.QAQI..VQ...E.K..GETVRIS.KAS..TF.TAGIQ   .V
AN10     MNFGFS.IF.VLVLK.VQCE.K.V...G.....GG.LK.S.AAS.FTFS.YAMS   .V

                40          52  53      60        70          82  83      90
                 |           ABC |       |         |           ABC |       |
ANO2     RQFPGNKLEWMGYMS    YSGSTRYNPSLRSRISITRDTSKNQFFLQLKSVTTEDTATYF
ANO1     ............IN    .D.RNN.....KN..............K............Y
ANO3     ............IN    .D.NNN.....KN..............K.N..........Y
ANO7     .....H.......IH    .....N.....K..............N..........Y
ANO5     K.R..QG...I..INP   NT.Y.V..QKFKDKATL.A.K.SSTAYM..S.L.SD.S.V.Y
ANO6     K.R..QG...I..INP   ST.Y.E..QKFKDKATL.A.K.SSTAYM..S.L.S..S.V.Y
ANO4     K.K..QG...I.WIYP   G..NNK..EKFKGKATL.I...SSTVYI..S.L.S....V..
ANO9     ..R..QG...I.EINP   SN.R.N..EKFK.KATLNV.K.SSTAYM.IS.L.S..S.V.Y
AN11     K.K..QG...I..INP   .NDG.K..EKFKGKATL.S.K.SSTAYIE.S.L.S..S.V.Y
AN12     K.K..QS...IAWIYA   GT.G.S..QKFTGKARL.V...SSTAYM.FS.L....S.I.Y
ANO8     QKM..KG.K.I.WINT   R..VPK.AEDFKG.FAFSLE..ASTAY..ISNLRND...A..
AN10     ..T.ERR...VASI.    SGYI.Y.PD.VKG.FT.S..NAR.ILY..MS.LRS....M.Y

                95   100               105  110
                 |    ABCDEFGHIJK      |    |
ANO2     CARGWP               LAYWGQGTQVSVSE
ANO1     ...EDDGYYI            FD.....STLT..S
ANO3     ...EGYGYF             FD......TLT..S
ANO7     ...VIYYYGSSYV        WF.......L.T..A
ANO5     ..YYGSS              YFD......TLT..S
ANO6     ..HYGRS              YFD......TLT..S
ANO4     .V.YGYDG              FG......L.T..A
ANO9     ....R.GSYVGG          F......NM.T..A
AN11     ...FGYYGR          YWYFDV..A..T.T..S
AN12     ...WD.INRG            F.......L.T..A
ANO8     .G.TDYYGST         YYAMD.....SS.T..S
AN10     ...WGHRYDVL             D......S.T..S
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FIG. 1. Deduced amino acid sequences of the *V* regions of the heavy chains of the anti-DNP-SL monoclonal antibodies AN01–AN12.

```
                -20       -10          1         10        20      27
                 |         |           |         |         |        ABCDEF
ANO2           MDFQVQIFSFLLISASVILSRGQIVLTQSPAIMSASPGEKVTMTCSASS
ANO1           ............M.....M...........L................
ANO3              ........M.....M...........L................
ANO9           ..............I..VM...EN........I...L......S.R...
ANO5             MRCSLQFLGVLMFWISGVS.D..I..DELSNPVAS..S.SIS.RSTKSLL
ANO6             MRCSLQFLGVLMFWISGVS.D..I..DELSNPVTS..S.SIS.RSTKSLL
ANO4             MR.LAELLG.LLFCFLGV.CD.QMN...SSL...L.DTI.I..H..Q
ANO8             MRF.VQVLG.LLLWISGAQCDVQI....SYLA.....TIIIN.R..K
AN11             MVFTPQILG.MLFWISA...D.........TL.VT..DS.SLS.R..Q
AN12     MHHTSMGIKMES..QV.VFVFLWLSGVD.D..M...HKF..T.V.DR.SI..K..Q
ANO7              MAW.SLI.SLL.LSSGAIS.A.V..ES. LTT....T..L..RS.N
AN10              MAW.SLI.SLL.LSSGAIS.A.V..ES. LTT....T..L..RS.T

                30        40        50        60        70        80
                 |         |         |         |         |         |
ANO2           SVYYMYWYQQKPGSSPRLLIYDTSNLASGVPVRFSGSGSGTSYSLTISRMEAEDAA
ANO1           ..S..F......R...KPW..L.........A...............S.......
ANO3           ..S..F......R...KPW.FL.........A....R..........S.......
ANO9           ..N..F.....SDA..K.W..Y.....P...A.........N.....S.AG....
ANO5     YK DGKT.LN.FL.R..Q..Q....LM.TR....SD.........DFT.E...VK...VG
ANO6     YK DGKT.LN.FL.R..Q..Q....LM.TR....SD.........DFT.E...VK...VG
ANO4        NINVWLS.......NI.K....KA...HT...S..........FT....SLQP..I.
ANO8        SISK.LA...E...KTNK....SG.T.Q..I.S.........DFT....SL.P..F.
AN11        SVSNNLH.F...SHE......KYA.QSI..I.S.........DFT.S.NSV.T..FG
AN12        DVSTAVA.......Q..K....SA.YRYT...D..T......DFTF...SVQ...L.
ANO7     GAVTTSN.AN.V.E..DHLFTG..GG.N.R.P...A.....LI.DKAA...TGAQT..E.
AN10     GAVT.SNSVK.V.E..DHLFTG..GGSN.R.P...A.....LI.DKAA...AGAQT..E.

                90   95          100   106 109
                 |    ABCDEF      |     A   |
ANO2           TYYCQQWSSYPP      ITFGVGTKLEL  KRA
ANO1           .........N..      ....S.....I  ...
ANO3           .......N.I..      ....A.......  ...
ANO9           ......FT.S.       S...A.......  ...
ANO5           V.....LVEF.       L...A.......  ...
ANO6           V.....LVEF.       L...A.......  ...
ANO4           ......GQ...       L.L.G.....I  ...
ANO8           H.....HNE..       Y...G.....I  ...
AN11           H.F...SN.W.       F...G.....I  ...
AN12           V...H.HY.S.       Y...G.....I  ...
ANO7           I.F.AL.Y.NH       LV..G....TVLGQP
AN10           V.F.AL.Y.NH       LV..G.A..TVLGQP
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FIG. 2. Deduced amino acid sequences of the *V* regions of the light chains of the anti-DNP-SL monoclonal antibodies AN01–AN12.